The University of Jordan

Authorization Form

I, Rana Atef Qasem , authorize the University of Jordan to supply copies of my Thesis/ Dissertation to libraries or establishments or individuals on request, according to the University of Jordan regulations.

Signature:

Date: 19-5-2011

الجامعة الأردنيــــة
كلية الدراسات العليا

التاريــخ: ١٩/ ٥ / ٢٠١١

نموذج رقم (١٨)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية
وقوانين الجامعة الأردنية وأنظمتها وتعليماتها
لطلبة الماجستير

أنا الطالب: رنا عاطف محمد قاسم الرقم الجامعي: (٨٠٩٠٥٥٣)

تخصص: التقنيات الحيوية الكليــــة: الزراعة

عنوان الرسالة:
Detection and identification of listeria monocytogens in white brined cheese using biochemical and molecular PCR techniques.

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير عندما قمت شخصيا" باعداد رسالتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الرسائل العلمية. كما أنني أعلن بأن رسالتي هذه غير منقولة أو مستلة من رسائل أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

توقيع الطالب: Ran qassim التاريخ: 2011/5/29

تعتمد كلية الدراسات العليا
هذه النسخة من الرسالة
التوقيع: ... التاريخ: ٢٠١١/٥/٢٩

DETECTION AND IDENTIFICATION OF *LISTERIA MONOCYTOGENES* IN WHITE BRINED CHEESES USING BIOCHEMICAL AND MOLECULAR PCR TECHNIQUES

By
Rana Atef Mohamed Qasem

Supervisor
Dr. Muhanad Walid Akash

Co-Supervisor
Dr. Hamzah Mari Al-Qadiri

This Thesis was Submitted in Partial Fulfillment of the Requirements for the Master Degree of Agricultural Biotechnology

Faculty of Graduate Study
University of Jordan

May, 2011

COMMITTEE DECISION

This thesis (Detection and Identification of *Listeria monocytogenes* in White Brined Cheeses Using Biochemical and Molecular PCR Techniques) was successfully Defended and Approved on the 11th of May, 2011

Examination Committee	Signature
Dr. Muhanad W. Akash, Chairman Assoc. Prof. (Plant Molecular Breeding)	....
Dr. Hamzah M. Al-Qadiri, Co-Supervisor Assis. Prof. (Food and Water Microbiology)	....
Dr. Ayed M. Al Abdallat, Member Assoc. Prof. (Plant Molecular Biology)	....
Dr. Ghadeer F. Mehyar, Member Assis. Prof. (Food Microbiology and Food Packaging)	....
Dr. Nasser M. Salameh, Member Assis. Prof. (Plant Molecular Genetics) Mutah University	....

Dedication

For dear God

Who gave me:

Loving Father Atef Mohamed Qasem for giving me the strength and moral support to complete this study

Loving Mother Maha Mohamed Qunbar for her great encouragement

Wonderful sisters Rania, Arwa, Dima and Hiba

Beloved husband Belal Al Hegaish

And Generous Doctors, Dr. Muhanad Akash and Dr. Hamzah Al Qadiri.

Acknowledgements

I would like to thank God for giving me the strength, confidence and hope to finish this research.

I would like to thank Dr. Muhanad Akash and Dr. Hamzah Al Qadiri for their help and encouragement.

I would like to thank my parents for their support, my wonderful sisters and my beloved husband.

I would like to thank all JFDA members: Eng. Khaled Qadoura and Dr. Rajab Al Najar for their support and facilitating my research.

I would like to thank food laboratory in Amman municipality for their great help and support to finish my work: Dr. Mervat Abbadi, Dr. Nasha`at Hatamleh, Eng. Sana Hazaymeh, Eng. Sana Awaysheh and Eng. Hana Frahaiat.

I would like to thank all my friends for their great help to complete my research: Shireen Qasrawi, Manar Rabba`a, Ayat Al Qura`an, Riyad Al Zamer, Isra`a Salem and Tamara Al Quadah.

Table of contents

List of figures

List of tables

List of appendices

List of abbreviations

Abbreviation	Symbol full name
ALOA	Agar *Listeria* according to Ottaviani and Agosti
α	Alpha
ATCC	American Type Culture Collection
Bp	Base pairs
ß	Beta
CFU	Colony Forming Unit
dNTPs	Deoxynucleoside Triphosphates
DNA	Deoxyribonucleic Acid
G+ve	Gram positive
HACCP	Hazard Analysis and Critical Control Points
H2O2	Hydrogen Peroxide
JFDA	Jordanian Food and Drug Administration
µg	Microgram
µm	Micrometer
mm	Milimeter
Min	Minute
%	Percent
PCR	Polymerase chain reaction
RTE	Ready to eat
R. equi	*Rhadococcus equi*
Rpm	Round per Minute
s	Second
Spp.	Species
S. aureus	*Staphylococcus aureus*
Taq	*Thermus aquaticus*
TSYEA	Tryptone Soya Yeast Extract Agar

DETECTION AND IDENTIFICATION OF *LISTERIA MONOCYTOGENES* IN WHITE BRINED CHEESES USING BIOCHEMICAL AND MOLECULAR PCR TECHNIQUES

By
Rana Atef Mohamed Qasem

Supervisor
Dr. Muhanad Walid Akash

Co-Supervisor
Dr. Hamzah Mari Al-Qadiri

Abstract

This study was conducted to detect and identify *Listeria monocytogenes* from white brined cheeses collected from Amman-Jordan using conventional and rapid biochemical methods, and definite confirmation using polymerase chain reaction (PCR) technique.
A total (250) samples (70 of Akkawi cheese, 63 of Haloum cheese, 40 of Pasteurized cheese, 45 of Nabulsi cheese and 32 of Boiled cheeses) were used in this study. The conventional International Organization for Standardization (ISO) - two stages isolation procedure was applied for pre-enrichment, enrichment of samples, and presumptive identification. Results of conventional method showed that out of total 250 samples, 28 (11.2 %) were found to be contaminated with *Listeria* species, with the highest prevalence (14.28 %) occurring in Akkawi samples. *Listeria monocytogenes* was isolated from 6 (2.4%) samples of the 250 samples examined. These six isolates were obtained from three Akkawi, one Haloum, one Pasteurized, one nabulsi and zero Boiled. Though using molecular method, the same six isolates were confirmed as *Listeria monocytogenes*. The results presented in this study indicated the potential risk of contamination of white brined cheeses products by *Listeria monocytogenes*.

1. Introduction

Listeria species are widely distributed in environment and can infect many types of animals such as domestic pets, livestock, rodents, amphibians and fish (Jalali and Abedi, 2008). It can be transmitted directly from animals to humans and this has been documented in veterinarians, farmers, and abattoir workers (Jalali and Abedi, 2008). *Listeria monocytogenes* is a catalase-positive, oxidase-negative, facultative anaerobic, gram-positive, rod-shaped foodborne bacterium (CDC, 2004). *L. monocytogenes* is considered as primary pathogenic to human (Klein and Juneja, 1997) and has a vital health concern due to its high mortality rate especially for immunocompromised people (i.e. pregnant women and neonates) (Koutsoumanis and Angeledis, 2007; European commission, 2005).

Infections by *L. monocytogenes* are the single biggest cause of foodborne disease listeriosis which causes diarrhea, nausea, vomiting and fever (Schuchat, *et al*., 1991). This bacterium has a very great ability to survive at low pH (2.5-5) and high salinity, additionally is considered as psycho tolerant microbe (Sado, *et al*., 1998). Fecal-oral transmission and food cross contamination are the probable means of infection and by which the approximate fatality rate is about 30% that may increase up to about 75% in high risk groups, such as pregnant women and immunosuppressed adults (Choi and Hong, 2003). Most documented cases of listeriosis appear to be foodborne, including those acquired during pregnancy. Different food items can be contaminated by *L. monocytogenes* particularly white brined cheeses as Akkawi, Nabulsi and boiled cheese (Rudolf & Scherer, 2001).

Between 1999 and 2001, about half of the outbreaks and sporadic cases of listerosis were associated with cheese and dairy products. The presence of *L. monocytogenes* in white brined cheeses is mainly environmental and this differs according to the production (Kabuki *et al*., 2004). *L. monocytogenes* is a bacterium pathogen that can presence in all dairy products, due to many factors, including its presence in processing facilities, it could survive from the control treatment procedures (water activity, acidity and refrigeration), and therefore may cause severe illnesses in selected groups of people (Sado *et al*., 1998). The most important characteristics of *L. monocytogenes* are the ability to grow in a wide range of environments as low as −0.4°C, resist heat, salt, nitrite, acidity, and can have a marginal survival rate when food preservation treatments are used to control this foodborne pathogen and to limit its virulence effect on human and animals (Sado *et al*., 1998).

The high prevalence of *L. monocytogenes* in food and the high fatality rate associated with listeriosis were considered as a public health hazard and causing loss to many processed food. The incidence of listeriosis as a foodborne illness in both industrialized and non-industrialized countries is increasing (CDC, 2002; Varnam and Evans; 1991 Fleming, *et al*., 1985); this is mainly due to the increase in food production, processing, storage and transportation (CDC, 1985). White brined cheeses are considered the most significant sources of this pathogenic bacterium due to its production from unpasteurized milk (Colak, *et al*., 2007). *L. mono*cytogenes growth depends on the conditions during manufacturing, ripening and storage of white brined cheeses. Among all the technological parameters that could affect the behavior of *L. monocytogenes* are

the nature and the activity of starter cultures, the rate and extent of white brined cheese acidification and humidity of white brined cheese during ripening (Vernozy-Rozand, 1998). Detection and identification procedures of *L. monocytogenes* from contaminated samples can be done using non-selective and selective enrichment culture media for recovery (24 to 48 hours) followed by biochemical identification tests. Recently, molecular PCR-based technique is more promising than the classical culture based technique and takes only a few hours (ISO 11290-1, 2004). Therefore shortening the time required for this pathogen identification and would be of great value for the food industry in Jordan. PCR-based verification technique is a widely used method to identify recovered bacterial cells in food products and which depends on the purity of the template target and efficient numbers of target molecule (ISO 11290-1, 2004).

Accordingly, this research work was principally carried out to:

1- Detect and identify *L. monocytogenes* in white brined cheeses marketed in Amman-Jordan using classical culture media and biochemical verification tests.

2- Identification of isolated *L. monocytogenes* using molecular PCR technique and confirmation of the results.

2. Literature review

2.1 The genus *Listeria*:

Listeria is a genus of bacteria that include six separate species*:* *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri,* and *L. grayi,* with only *L. monocytogenes* and *L. ivanovii* are considered virulent. Human cases of *L. ivanovii* infection are rare, whereas *L. monocytogenes* is an important foodborne pathogen (Swaminathan, 2001).

The first published report about *L. monocytogenes* strain was first identified and classified in 1926 an outbreak in rabbits and this was published by Murry *et al*., in 1926 (CFSAN, 1992), but has only gained interest development by the U.S. federal regulatory bodies. The author named this microorganism *Bacterium monocytogenes* according to the particular production of mononuclear leucocytosis. In 1927, Pirie discovered a new microorganism agent of what he mentioned the Tiger River disease (Pirie, 1927). He named the bacterium to the honor of Lord sister, a British famous bacteriologist *Listerella hepatolytica*. Murray and Pirie then agreed to call this pathogen bacterium "*Listerella monocytogenes*" (Murray, 1926) *L. monocytogenes* can be found in the gastro-intestinal tract of various several animal species and humans. It has believed to be present in up to 10 percent of humans (CFSAN *et al.,* 1992). *L. monocytogenes* is the primary causative agent of listeriosis. Listeriosis can be classified under two categories: invasive and non-invasive. Invasive listeriosis is the severe form of the disease which typically characterized of incubation period of two to three weeks, but can extend up to three months. (CFSAN *et al*.1992).

Non-invasive listeriosis causes gastrointestinal illness, which might include diarrhea, headache, abdominal pain and cramps, nausea, vomiting and fatigue (CFSAN *et al*. 1992).

L. *monocytogenes* is a non-saprogenic, facultative anaerobic and that has a great ability to survive on high and low temperatures (Sado, *et al*., 1998). The main source of *L. monocytogens* in milk and milk products particularly white brined cheeses, this is probably according to fecal contamination (Griefiths, 1989; Gameiro, *etal.,* 2007), additionally, *L. monocytogenes* may present in raw milk in numbers of count approximately 100 CFU/ml (Griefiths, 1989). *L. monocytogenes* may be found in animal feed, it has been reported as a source of foodborne disease listeriosis in many cases in animal farm (Fenlon, 1999). *L. monocytogenes* can be present in white brined cheeses and can be varied from 0.5 to 46.0% (Guerra, *et al.*, 2001; Manfreda, *et al*., 2005; Pintado, *et al.*, 2005; Colak, *et al*., 2007). In 1999, The State Veterinary Institutes in the Slovak Republic have been reported the occurrence of *L. monocytogenes* strains which can lead to listeriosis, a total number 6247 of samples of milk and milk products were analyzed and showed 160 positives. *L. monocytogenes* was the most reported pathogen in raw milk samples represented approximately 7.56% and of the cheese curds representing approximately 5.43 % (Pastorova, *et al*., 2001).

2.2 Microbiology:

L. monocytogenes is facultative anaerobic, Gram-positive, motile bacillus. This bacterium grows well on a majority of common media. It can grow well in broth and on blood agar media; some species produce a narrow zone of beta-haemolysis. (Klara and Ellen, 2009).

However, media that contains fermentable carbohydrate, such as glucose, improves its growth. (Rocourt, 1999). *L. monocytogenes* is a psychrotrophic pathogen and capable of growing at refrigeration temperatures (Jay, 2000). This bacterium is motile when grown at 20 to 25°C (Rocourt, 1999). *Listeria* is not a spore forming bacterium; however it is moderately tolerant to freezing, drying, as well as heating (FDA, 1999).

2.3 Natural Environment:

L. monocytogenes is considered to be in water, soil, and plant material (Fenlon, 1999). This bacterium has also been found in animals feed. However, due to the low water activity of feeds, its presence in feed is not considered to be of high concern which prevents growth of the bacterium (Fenlon, 1999). *L. monocytogenes* can present in the fecal material of a large variety of healthy animals. *L. monocytogenes* is a non-spore forming bacteria, that would has the ability to survive moderately harsh environmental conditions, more so than other non-spore forming bacteria. Having this ability as well as being able to survive on processing equipment, makes *L. monocytogenes* a serious threat to the food industry (Fenlon, 1999).

2.4 Epidemiology:

Listeria spp. are distributed worldwide, but human illness was reported in developed countries. *Listeria* spp. are considered to be an important cause of zoonoses, infecting many types of animals (Schuchat *et al.*, 1991). In mammals, *L. monocytogenes* can cause abortions and is the main cause of meningitis (Jacquet *et al.*, 2002). Fecal-oral transmission is the probable means by which organisms are spread in animals. Transmission of the pathogen can be directly from animals to humans. Vertical transmission from mother to neonate occurs

trans placentally (Jacquet *et al*., 2002). Most of the listerosis cases appear to be outbreak, including those acquired during pregnancy. listeriosis was first reported in 1981, numerous foodborne outbreaks of *L. monocytogenes* have been reported worldwide. It was the cause of an estimated 2500 cases of meningitis, encephalitis, fetal death, and prematurity and 500 deaths in the United States annually (Jacquet *et al*., 2002). The infectious dose was estimated to be 104-106 CFU/g but may be lower in immunocompromised patients. (Schlech, 1997).

Table 1: Overseas prevalence and quantitative data for *L. monocytogenes* .

Country/ Region	Cheese Type	No. samples tested	No. (%) positive for L. monocytogenes	Reference
Australia	Soft cheeses Soft cheeses	437 - U 28 - U	15 (3.4) 1 (3.6)	Arnold and Coble, 1995
Brazil	(Brazilian soft white cheese)	17 R 33 U 53 U	7 (41.1) 1 (3.0) 3 (5.7)	Da Silva et al., 1998
Canada	(Soft cheese)	182 – U 192 - U	0 (0) 2 (1.0)	Farber et al., 1987
Chile	Soft cheese	256 - U	2 (0.8)	Cordano and Rocourt, 2001
Costa Rica	Soft cheese	20 - U	9 (45.0) no values	Monge et al., 1994
England	Soft cheeses	251 - U	1 (0.4)	MacGowan et al., 1994
England and Wales	soft ripened cheese	769 366	63 (8.2) samples <500/g	Greenwood et al., 1991

U=Unknown, P=Pasteurized, R=Raw.

(CDC, 2002)

2.5 Transmission:

Endogenous transmission may occur from the gastrointestinal tract of a carrier who is immunocompromised and is slightly would have septicemia and meningitis (Mead *et al*., 1999).

Exogenous infection can occur from the infected female genital tract and produce disease to new born (Winter *et al*., 2004).

2.6 Detection Methods

2.6.1 Conventional cultural method:

The detection and identification of *Listeria* spp. might be difficult because of its existence in low numbers along with high number of competing microorganism. Cultural methods are used for detection *L. monocytogenes* in food and environmental samples and they based on isolation protocols using selective agars and confirmation tests by biochemical reactions. Selective media is used for *Listeria* isolation include Oxford, and Modified Oxford agars. Conventional techniques, using cultural and biochemical assays, require at least 4-5 days for complete *listeria* identification; these methods are very specific and sensitive however the realization time is too long (Sado *et al*., 1998 and Gasanov *et al*., 2005)

2.6.2 PCR-based detection of *L. monocytogenes*

In the past years, biotechnology researches have reported the development of rapid methods that reduce analysis time and offer great sensitivity and specificity in the detection of pathogens. PCR is one of the most important techniques that have been used for rapid identification of microorganisms in food products, however, PCR benefit in detection microbes in food samples is limited due to the inhibitory factors as the isolation procedures for bacterial

target DNA, and by dairy product components, that may present in some instances (Powell, *etal.,* 1994; Wernars, *etal.,* 1991). The development of PCR technique has the concern of the development of rapid, specific and sensitive methods for the detection of food borne pathogens (Powell, *etal* 1994; Rossen, 1988; Wernars, *etal* 1991).

Data indicate that the infective dose of *L. monocytogenes* in contaminated food samples could be less than 100 CFU/g (Swaminathan, 2001). Detection and isolation of *L. monocytogens* remains complicated due to the inability to find single procedure to identify *L. monocytogens* in all types of food (Donnelly, 1999). Several studies (Lawrence and Gilmour, 1994; Lampel*, et al*., 2000; Almeida and Almeida, 2000; Holko*, et al*., 2001) have been conducted using PCR for rapid identification of *L. monocytogenes* in milk and milk products. The objective of Holko (2001) experiment was to analyze 100 various samples by traditional cultivation methods and compare the results with those by nested PCR. Eighteen positive and 72 negative samples were detected by both methods. However, the PCR method yields results within 2 days. For PCR detection, two pairs of primers (PRFA 1 and 2, LIP 1 and 2) were used with affinity to *prfA* gene. The size of the PCR product was 1060 bp fragment in a first step of PCR and 273 bp fragments in nested PCR. They concluded that PCR was employed to solve the problem of interpretation of classical biochemical and serological typing in only one step without the need for additional examination (Holko, *et al*., 2001).

2.6.2.1 *Hly* A gene in *Listeria monocytogenes*:

L. monocytogenes is the major foodborn pathogen; in 1980s was the start of investigations into the molecular mechanisms underlying *Listeria* virulence genes. The attention of researchers was first show the importance of hemolytic activity, these studies was between 1986 and 1989 to the discovery of the hemolysin gene, *hly*, and to elucidation of the key role that hemolysin plays in escape from destruction inside phagosomes, a prerequisite for intracellular bacterial proliferation. The *hlyA* gene encodes listeriolysin O, which is an essential virulence factor was sequenced and has a GC content of 36% (Mengaud *et al.*, 1988). The *hlvA* determinant encodes for a secreted protein of 504 amino acids. In a study to analyze the structural chromosomal region containing *hlyA* and evaluate the role of listeriolysin in pathogenicity was the first genetic experiment to correlate the hemolytic phenotype with virulence in *L. monocytogenes* and were performed by Gaillard *et al*. (Gaillard, 1986). Using transposon mutagenesis, they obtained a Hly- mutant which was virulent. By cloning and sequence analysis, they showed that the transposon had inserted in *hlyA* (Mengaud, 1988). This demonstrated that the region containing hlyA played a crucial role in virulence.

2.7 *Listeria monocytogenes* in white brined cheeses:

There are a great number of cheeses that are classified under the category of white brined cheeses (U.S. Department of Agriculture, 1995). White brined cheeses include cottage cheese, cream cheese, Haloum (Ayto, 1990), Akkawi, Boiled cheese and Nabulsi white brined cheese (Tamime, 1991). All the initial processes for white brined cheeses making are similar. Milk is pasteurized by using standard methods, and then concentrated through acidification. White

brined cheeses characteristics are perfect for *L. monocytogenes* growth because they are slightly acidic, have high moisture content and high water activity, and have high fat content which can play a protective role for the organism against control treatments, and also because they contain high amounts of available nutrients. A number of surveys was done, estimated the incidence of *L. monocytogenes* in raw milk have been made in various countries such as Canada, France, Netherlands, Spain, and USA (Carlos *et al*., 2001). The first outbreak of listeriosis reported in USA was associated with Mexican-style cheese in California (James *et al.*, 1985). Surveys in many several countries estimated that *L. monocytogenes* is a common contaminant of white brined cheese and semi hard cheese (Gameiro, 2007).

In a study of detection *L. monocytogenes* from Italian cheeses (mozzarella, crescenza and ricotta from bovine milk) from local stores, a set of primers including L1 (5′GTGATAAAATCGACGAAAATCC3′) and L2 (5′CTTGTAAAACTAGAATCTA GCG-3′) specific to the coding region of the *actA* gene were used (Vazquez-Boland, et al., 1992). The procedure was applied to contaminated Italian cheeses and found low levels of *L. monocytogenes* in mozzarella and crescenza (4 CFU g) whereas in ricotta the detection limit was higher (40 CFU g) (Longhi, 2003).

Listeria spp. have been found in various foods, including unprocessed foods of animal origin like fish (Davies *et al*., 2004). Seafood products including RTE(Ready To Eat) products such as smoked salmon, shrimp, rainbow trout, crawfish and fish salad had also been found to be contaminated with *L. monocytogenes* (Choa *et al*., 2006).

2.8 Prevalence of *L. monocytogenes* in food:

Because *L. monocytogenes is* ubiquitous in nature, its transmission was not always definitively known. Since the 1980's, it has been determined by epidemiologic and laboratory surveillance of contaminated cheese and raw vegetable outbreaks, that listeriosis can be transmitted by consumption of several contaminated foods samples, table:2 (Schlech *et al*., 1983). Although *L. monocytogenes* relationship between the environment, animals, humans, and food is still not absolutely clear, *L. monoctogenes* as a foodborne pathogen brought the organism to the forefront of food safety and regulatory issues. *L. monocytogenes* can be found in different uncooked foods, such as cheese, meat, and vegetables as a result of contamination of food during production and distribution (Bula *et al*., 1995).

In food industry, *L. monocytogenes* is able to colonize contact surfaces, creating biofilms that are difficult to be removed. Because contamination of food products in transporting, cutting and chilling areas is mainly due to cross contamination, therefore, it is important not only to prevent, but also to know the prevalence of *L. monocytogenes* in different points of the food production chain, from the raw material to consumption (Vitas *et al*., 2004).

Table 2. Out breaks due to *Listeria monocytogenes*:

Location (year)	No. of cases (No. of deaths)	No. perinatal / No. nonperinatal	Foods associated
Boston (1979)	20 (5)[a]	0/20	Raw celery, tomatoes, lettuce [b]
New Zealand (1980)	29 (9)	22/7	Shellfish, raw fish [b]
Maritime Provinces (1981)	41 (17)	34/7	Coleslaw
Massachusetts (1983)	49 (14)	7/42	Pasteurized milk [b]
California (1985)	142 (48)	93/49	Jalisco cheese
Canton de Vaud, Switzerland (1983-1987)	122 (31)	63/59	Raw milk, cheese
Philadelphia (1986-1987)	36 (16)	4/32	Ice-cream [b], salami [b]
Connecticut (1989)	9 (1)	2/7	Shrimp [b]
United Kingdom (1987-1989)	>300	NK [d]	Pâté [b]

[a] For two of these five deaths, an underlying disease, not listeriosis, was apparently the cause of death.
[b] Foods only epidemiologically linked.
[d] NK, not known.
(Source: Farber *et al*., 1991)

3. Material and Methods

3.1 Reference bacterial strains:

The reference bacterial strain used in this study was *Listeria monocytogenes* ATCC 7644 (Remel, USA) as positive control. *Staphylococcus aureus* ATCC 25923 and *Rhadococcus equi* ATCC 6939 (MicroBiologics, USA) were also used to undertake the CAMP (Christie, Atkins, Munch-Petersen) test.

3.2 Sample collection:

Two hundred fifty (250) white brined cheese samples were randomly collected from different local grocery stores located in Amman during the period of January-March, 2011 according to production date September 2010, temperature degree of the white brined samples ranging from 0-10 C and salinity percentage ranging from 4% - 7%. The collected white brined cheeses included seventy (70) samples of Akkawi s, sixty three samples (63) Haloum, forty five (45) samples Nabulsi, forty (40) samples of pasteurized and thirty two (32) of boiled cheese.

White brined cheese samples were taken from three different points of each product by using sterile surgical forceps and immediately placed in sterile stomacher bags. Samples were transported to laboratory under aseptic and refrigerated conditions in portable insulated cold-boxes. Samples were kept at 4° C and analyzed within 24 hours.

3.3 Detection and isolation of *Listeria* species by conventional method

3.3.1 Culture media

Several culture media were used for the detection and isolation of *L. monocytogenes* from each specimen and which included the following: Half Fraser broth (Oxoid, UK); Fraser broth (Oxoid, UK); chromogenic *Listeria* agar (Oxoid, UK); Listeria selective agar (Oxford Formulation) (Oxoid, UK),

tryptone soya agar (Oxoid, UK), tryptone soya broth (Oxoid, UK); yeast extract powder (Oxoid, UK), sheep blood agar base (Oxoid, UK), and motility agar (Hi media, India).

L. monocytogenes was isolated according to the ISO standard (ISO 11290-1, 2004 amendment 1, (Modification of the isolation media and the haemolysis test, and inclusion of precision data), this method involves two stages for pre-enrichment and enrichment of the samples to selectively resuscitate the injured *L. monocytogenes* cells. According to this method, a 25 g representative portion from each white brined cheese sample was introduced aseptically into a sterile stomacher bag containing 225 ml of Half Fraser Broth (primary enrichment medium) to obtain a 1:10 sample dilution. The samples were then homogenized for 1 minute at 260 rpm in a stomacher circulator followed by incubation for 24 hours at 30ºC.

After incubation, 0.1 ml sub-sample from each Half Fraser broth culture was added to 10 ml of Fraser broth (secondary enrichment medium), and incubated for 48 hours at 37 ºC. A loopful of the Fraser broth enrichment culture was streaked on the surface of chromogenic *Listeria* agar and on *Listeria* selective Agar (Oxford Formulation). These selective agars were then incubated for up to 48 hours at 37 ºC. Selective agars were observed for suspected (typical) colonies at 24 hours and 48 hours of incubation. The suspected typical *Listeria* cells were those that appeared grayish colonies surrounded by black halos and sunken centers, these are called presumptive *Listeria* colonies Whenever possible, up to 5 suspected colonies showing typical morphology of *L. monocytogenes* on these isolation media were selected and further examined for identification process.

3.4 Identification of *Listeria* spp.

The suspected colonies were streaked onto tryptone soya agar supplemented with 0.06% of yeast extract agar (TSYEA) and incubated at 37 ºC for 24 hours.

The following tests were used for identification process:

3.4.1 Catalase reaction

One drop of 3% hydrogen peroxide (H_2O_2) was placed on bacterial colony on a clean microscopic slide. Positive reaction was indicated by formation of oxygen gas bubbles (FDA Bacteriological Analytical Manual, 1998).

3.4.2 Oxidase test

A small portion of the bacterial colony was placed on Oxidase strip (Oxoid, MB0266A). Positive result was indicated by immediate change in color to deep blue/violet color (FDA Bacteriological Analytical Manual, 1998).

3.4.3 Motility test

An inoculating needle was used to stab the motility agar with suspected colonies (Himedia, M260) and incubated up to 5 days at 25 ºC. *Listeria* spp. were motile, giving a typical umbrella-like growth pattern (ISO 11290-1, 1996).

3.5 Confirmation of *L. monocytogenes*

3.5.1 Haemolysis test

Sheep blood agar base supplemented with 7% sterile sheep blood was stabbed by using an inculcating needle (Oxoid, CM0854), and with a typical colony from TSYEA and then incubated at 37 ºC for 24 hours (ISO 11290-1, 1996). After incubation, positive and control result (*L. monocytogenes*) showed narrow, clear and light zones (ß-haemolysis), while negative and control result (*L. innocua*) showed no clear zone around the stab (ISO 11290-1, 1996) .

3.5.2 CAMP (Christie, Atkins, Munch-Petersen) test

Staphylococcus aureus and *Rhadococcus equi* cells were inoculated into a sheep blood agar plate by making a narrow streak down to the center of the plate with a loop or the edge of a needle. The test organism was streaked in straight-line inoculums at right angles to the *S. aureus* and *R. equi*. The plates were incubated at 35 °C for 24 hours. Incubation in ambient air is recommended to reduce the number of false positives results. An enhanced zone of ß-haemolysis at the intersection of the test cultures with each of the cultures of *S. aureus* and *R. equi* was considered to be a positive reaction. The positive reaction with *R. equi* was seen as a wide (5 mm to 10 mm) "arrow-head" of haemolysis. The reaction was considered as negative if the zones are weak and extended only about 1 mm at the intersection of the test culture with the diffusion zone of the *R. equi* culture. A positive reaction with *S. aureus* appeared as a small zone of enhanced haemolysis extended only about 2 mm from the test culture (ISO 11290-1, 1996).

3.5.3 Carbohydrate utilization

The Microbact™ Listeria 12L Kit System (Oxoid, MB1128A) was used for the rapid biochemical verification. This identification kit system is a standardized micro-substrate system designed for identification of *Listeria* spp. Each identification strip consists of 12 verification tests, (11 sugar utilization tests (Esculin, Mannitol, Xylose, Arabitol, Ribose, Rhamonse, Trehalose, Tagatose, Glucose-1-Phosphate, Methyl-D-Glucose, and Methyl-D-Mannose) in addition to a rapid haemolysis test. The reactions occurring during the incubation period were estimated by whether changing in the color or the blood sheep cells have

been lysed. The results were analyzed by Microbact software (Oxoid, MB1244A) to determine the percent probability number of *L. monocytogenes*.



Figure 1: Microbact™ *Listeria* 12L Identification Kit

3.6 Molecular identification techniques:

3.6.1 DNA extraction

Genomic DNA was extracted according to the boiling method (Bansal, 1996). Bacterial pellets were washed with 1ml phosphate buffer saline (PBS) to dilute substances. PBS was used as diluents to dry biomolecules and it can structure around the substance to be immobilized to a solid surface (Dulbecco, *et al.*, 1954). PBS is a modified buffered substrate used in cold water and can recover serotypes of bacterial cells from food (Dulbecco, *et al.*, 1954). The thin film of water that binds to the substance prevents denaruration or the incidence of conformational changes, sample were then centrifuged at 10000 rpm for 4 min, resuspended in the same volume of cold water and incubated in heat block, Genomic DNA was obtained after 10 min at 95C.

3.6.2 PCR procedure:

Specific *L. monocytogenes* primers were selected based on the published nucleotide sequence of the *hlyA* gene (Mengaud *et al.*, 1988). A pair of primers, forward primer (5'-CATCGACGGCAACCTCGGAGA-3'), and reverse primer (5'-ATCAATTACCGTTCTCCACCATT-3). The method, as described by Jalali & Abedi (2008), was followed for amplification of a 417 bp internal fragment of the *hylA* gene. A 25µl of PCR reaction containing 12.5µl PCR blue Master Mix solution, 2X (iNtRON-MAX II), 1µl of each primer at 5 pmole in 9 µl of Nuclease Free Water (NFW) and 1.5 µl of each DNA was used.

Thermo cycling conditions included an initial hold of 5 min at 94ºC, then denaturation step at 94º C for 45 s, annealing at 58º C for 45 s and a 45 s extension at 72 º C for a total of 35 cycles. A final extension at 72º C for 7 min followed a final hold at 4º C.

3.6.3 Detection of amplified PCR products

Agarose gel electrophoresis was performed as described by (Sambrook *et al.* 1989). It was added to TBE (Tris Borate EDTA) buffer and heated in a microwave oven until the agarose dissolved. The agarose solution was cooled down to 60 ºC, and Redsafe TM nucleic acid staining solution, as DNA visualization agent, was added to the solution and mixed thoroughly. The solution was poured into the mold to make an agarose gel 5 mm thick, and the comb was placed 1.0 mm above the plate. After the gel was completely set for 45 min at room temperature, the comb was removed to make a well for sample loading. The gel was put into the electrophoresis unit and the electrophoresis buffer was added to cover the gel to a depth of about 1 mm. (Sambrook and Russell, 2000).

A 15 µl of each amplified PCR products were electrophoresed on 2% agarose gel using 1X TBE running buffer, (Zhou & Jiao, 2005; Jalali & Abedi, 2008). The PCR products were visualized with UV transilluminator and photographed with the gel documentation system (Gel Doc 2000, BIO-RAD, USA). The DNA standard of 100 bp DNA ladder (0.1µg/µl) was used to determine the size of the amplified fragments..The size of the tested DNA fragments can be determined by comparing it with the size of the DNA bands in the DNA ladder and on a piece of semi-log graph paper, the log of DNA ladder band sizes can be plotted each against their distances traveled from the well. A line can be drawn connecting all points, from this line; it should be possible to determine the molecular size of the unknown DNA bands.

4. Results

4.1 Growth on selective media

The tested reference strain of *L. monocytogenes* (ATCC 7644) showed typical morphology of *Listeria* species on Oxford agar (OXA) as grayish colonies surrounded by black halos and sunken centers, additionally, the reference strain showed green-blue colonies surrounded by halo zone on Chromogenic *Listeria* agar (ALOA). The Suspected *Listeria* spp. isolates recovered from white brined cheese samples showed variable morphological characteristics on both Oxford agar (OXA) and Chromogenic *Listeria* agar (ALOA). Results were showed on table 3 and in (Fig. 2).

Table 3: Growth on OXFORD and ALOA agars.

Number of isolates	Growth on OXFORD	Growth on ALOA
Reference *L. monocytogenes* strain	Gray colony ,sunken center with blackening	Green colony with halo
Akkawi (3)	Gray colony ,sunken center with blacking	Green colony with halo
Haloum(1)	Gray colony ,sunken center with blacking	Green colony with halo
Boiled (0)	No growth	No growth
Nabulsi (1)	Gray colony ,sunken center	Green colony with halo
Pasteurized (1)	Gray colony ,sunken center	Green colony with halo

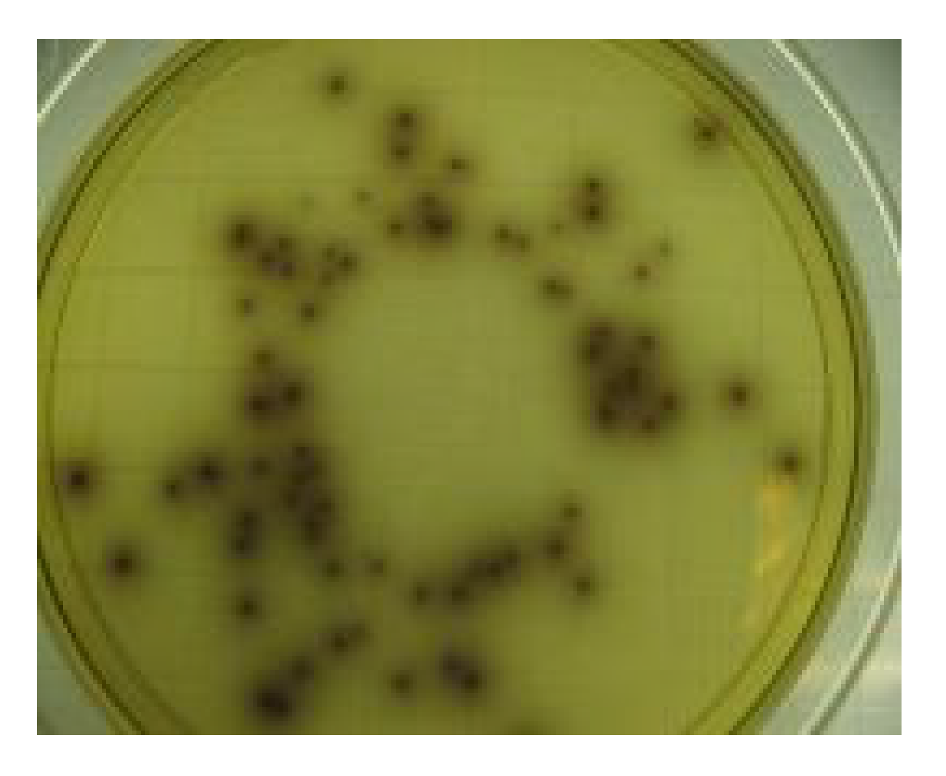


Figure: 2 Colonies of typical *Listeria monocytogenes* as they appear when grown on *Listeria* selective agar.

4.2 Morphological studies:

The characteristics of typical reference strain *L. monocytogenes* colony (ATCC 7644) on TSYEA agar were circular colonies, 1-1.5 mm in diameter, and showed an entire edge convex (Fig. 3).

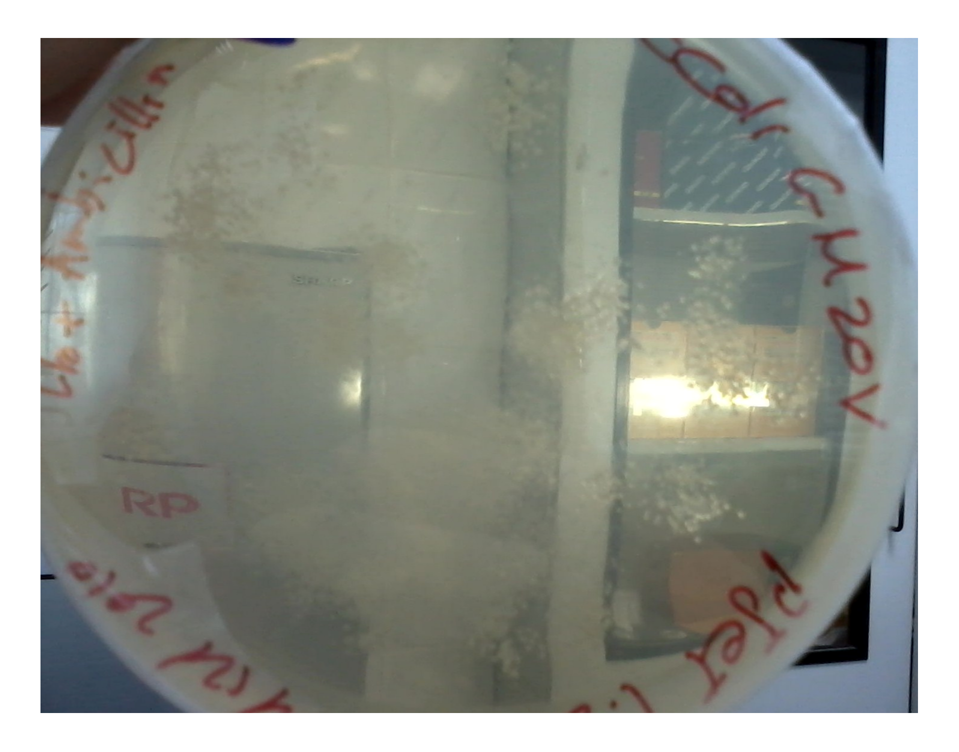


Figure: 3 Typical colonies of *Listeria monocytogenes* as they appeared when grown on *Listeria* TSYEA

4.3 Catalase and Oxidase examinations

The tested reference strain *L. monocytogenes* (ATCC 7644) is catalase positive and oxidase negative. Suspected *Listeria* spp. isolates recovered from white brined cheese samples showed variable reactions with catalase and oxidase tests. The results are shown in table 4.

Table 4: Catalase and oxidase tests results:

No. of isolates	Catalase test	Oxidase test
Listeria monocytogens strains	Positive	negative
Akkawi (3)	Positive	negative
Haloum (1)	Positive	negative
Nabulsi (1)	positive	negative
Pasteurized (1)	positive	negative
Boiled (0)	negative	negative

4.4Biochemical confirmation of suspected *Listeria* species

Based on the results obtained from the growth on selective media, biochemical examinations of oxidase test, catalase test, and motility test; 78 out of 250 samples were presumptively isolates. These isolates were recovered from 70 samples of Akkawi cheese, 63 of Haloum cheese, 32 of boiled cheese, 40 of pasteurized cheese, 45 of Nabulsi cheese, and results shown in Table 5.

Table 5: Types and numbers of tested samples, and number and percentages of presumptive and confirmed *Listeria* species using biochemical tests for white brined cheese samples collected from local grocery stores in Amman:

Type of white brined cheese	Number of tested samples	Number of presumptive isolates and (%)	Number of confirmed *Listeria spp*. and (%)
Akkawi	70	34 (48.5 %)	10 (14.28 %)
Haloum	63	11(17 %)	7(11.1)
Pasteurized	40	10(25 %)	4 (10 %)
Nabulsi	45	13 (28.8 %)	5 (11.1 %)
Boiled cheese	32	10 (31.2 %)	2 (6.25 %)
Total	250	78 (31.2 %)	28 (11.2 %)

4.5 CAMP test.

Out of 78 suspected *L. monocytogenes* isolates recovered, only 6 isolates were CAMP test positive (Table 6).

Table 6: Results of suspected *L. monocytogenes* reaction with CAMP test

Camp		**Sample Type**	**No. of isolates**
R.equi	*S. aureus*		
-	+	American Type Culture Collection (ATCC)	*Listeria monocytogenes*
-	+	(3)	Akkawi (3)
+	-	(1)	Haloum (1)
-	+	(1)	Pastrurized (1)
-	-	(1)	Nabulsi (1)
-	-	(0)	Boiled (0)

-: no reaction

+ : > 90% positive reaction

4.6 Rapid biochemical tests (Microbact™ *Listeria* 12L Kit):

The tested reference strain *L. monocytogenes* (ATCC 7644) utilized arabitol, rhamnose, trehalose, methyl-d-glucose and methyl-d-mannose, hydrolyzed esculin and lysed RBCs (blood cells). However, suspected *Listeria* spp. isolates recovered from white brined cheeses products showed variable biochemical reactions with rapid biochemical test (Microbact™ *Listeria* 12L Kit). Out of 78 suspected isolated *Listeria* spp. only one isolate out of six isolates was detected by rapid biochemical test as one *L. monocytogenes* and the other isolates were distributed between *L. ivanovii* (3 isolates), *L. grayi* (3 isolates)

Table 7: Results of the rapid biochemical test using Microbact™ *Listeria* 12L Kit on suspected *Listeria* spp. isolates recovered from white brined cheese.

Listeria spp	No. of isolates	No. Correctly Identified by Microbact (24h)	% Correctly Identified
L. monocytogenes	1	1	99
L. ivanovi	3	3	99
L. garyi	3	3	80

4.6 Definitive confirmation of *L. monocytogenes* using PCR molecular technique.

4.6.1 PCR molecular technique targeting *hlyA* gene:

Primers that were used in this research (5'-CATCGACGGCAACCTCGGAGA-3') and (5'ATCAATTACCGTTCTCCACCATT-3') are based on the amplification of a segment of the *hly* A protein gene. The primers amplified DNA sequence has 417 base pairs of nucleic acids. A positive PCR test will result in a fluorescence response produced within 35 cycles of the PCR amplification. A negative PCR test will normally not produce visible fluorescence. If fluorescence occurs above the positive control value, the results of the test are invalidated and the analysis must be repeated with precautions taken to eliminate possible sources of error. The 417 bp product amplified by the primer sets for all 78 presumptively isolates, six isolates were identified as *L. monocytogenes* (Fig 4 and Fig 5). Boiled cheese samples were free from *L. monocytogenes* (Fig 6). Three samples of Akkawi cheese showed positive *L. monocytogenes,* and only one isolate of *L. monocytogenes* from each Nabulsi, Haloum and pasteurized cheeses.

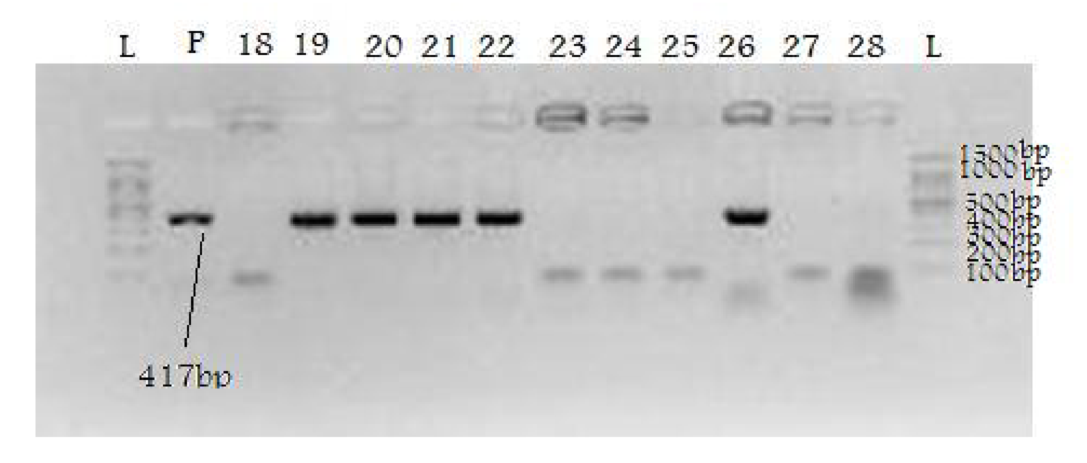


Figure:4 Agarose gel electrophoresis (2%) of Akkawi cheese, pasteurized cheese and Nabulsi. L: DNA marker, P: positive control, lane 19, lane 20 and lane 21 show positive *L. monocytogenes* recovered from Akkawi white brined cheese. Lane22 shows positive *L. monocytogenes* of pasteurized cheese, lane 26 show positive *L. monocytogenes* recovered from Nabulsi cheese

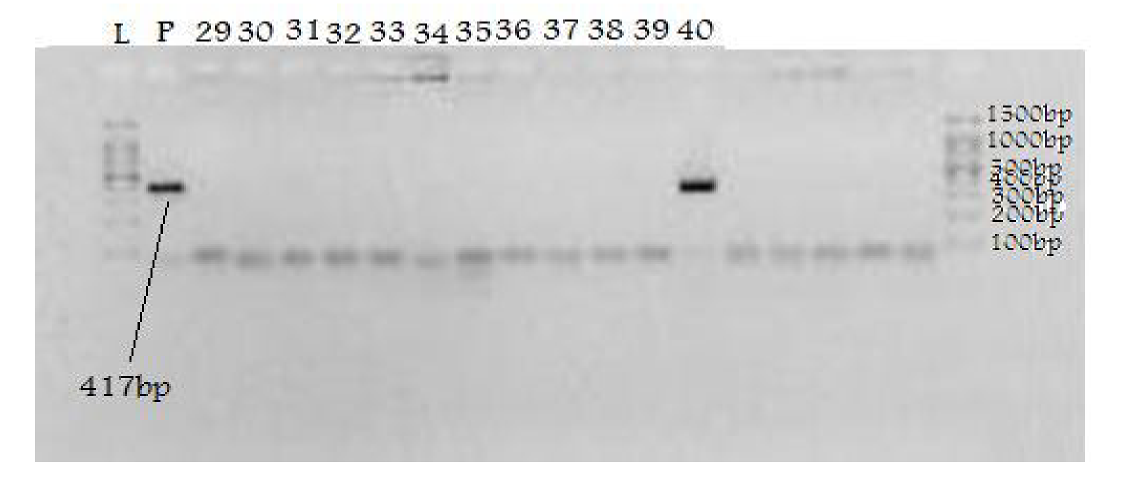


Figure: 5 Agarose gel electrophoresis (2%) of Haloum cheese. L: DNA marker, P: positive control, lane 40 show positive *L. monocytogenes* recovered from Haloum cheese.

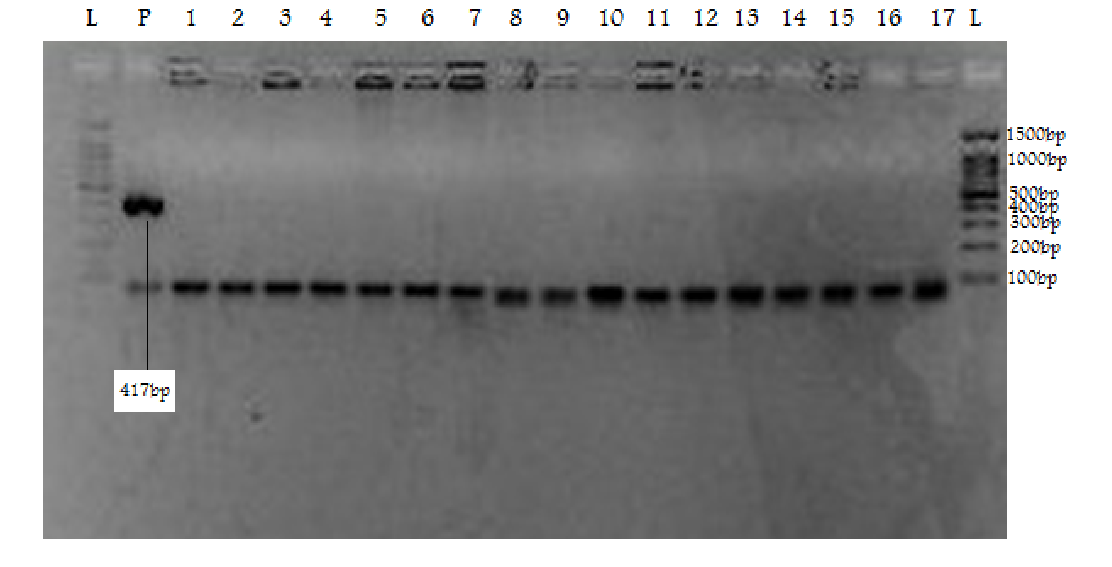


Figure: 6 Agarose gel electrophoresis (2%). L: DNA marker, P: positive control, from lane1 to lane 17 show *negative Listeria monocytogenes* of boiled **cheese**

Table 8: Prevalence of *L. monocytogenes* in white brined cheese using conventional and PCR techniques.

Cheese sample type	No. of sample tested	biochemical methods No.	PCR assay
Akkawi	70	3	3
Haloum	63	1	1
Pasteurized	40	1	1
Nabulsi	45	1	1
Boiled cheese	32	0	0

5. Discussion

Few studies on detecting *L. monocytogenes* from white brined cheeses and other food samples were conducted worldwide. The real situation of foodborne listeriosis in Jordan is unknown, and little information exists on the prevalence of *L. monocytogenes* in white brined cheese products. The eating habits of Jordanian people are also different from those of western countries. The first step to show the importance of *L. monocytogenes* in white brined cheese products is to provide data on prevalence of the bacterium in these food products.

The standard method for isolation and detection of *Listeria* spp. in food samples is the use of the enrichment procedure followed by selective media (Vlaemynck *et al*. 2000 and Beumer and Hezelger, 2003). ALOA medium has proved to be a useful and significantly better assay than other media for the isolation and differentiation of *L. monocytogenes* from non-pathogenic *Listeria* species, because *L. monocytogenes* colonies on ALOA agar exhibited clear halo zone that gave same positive results by using PCR technique (Vlaemynck *et al*., 2003; Beumer and Hezelger 2003). The detection of pathogenic *L. monocytogenes* by this media involves cleavage of the substrate, L-α-phosphatidyl-inositol by the virulence factor phosphatidylinositol-phospholipase-C (PI-PLC) and phosphatidylcholin-phospholipase-C (PC-PLC) produced by pathogenic *L. monocytogenes* resulting in the formation of a white precipitation zone (halo) around the colony. Souza (2002) detected 1.40% of *L. monocytogenes* of 70 samples of hand-made sour cheese sold in Fortaleza– CE, while Ramos and Costa (2003) detected 1.70% in samples of the same kind of cheese sold in Manaus. Oliveira (1993) detected 2.00% of *Listeria monocytogenes* in Brazilian Minas cheese in the retail business of Goiânia – Goiás. This percentage was also found by Schwab, in 1994, in colonial hand-made cheese

samples sold in Porto Alegre and this might be for cross contamination. White brined cheeses can be infected with *L. monocytogenes* by the cross contamination of raw materials or lack of hygienic practices and aseptic conditions during manufacturing, storage and distribution (Vlaemynck *et al*., 2003; Beumer and Hezelger, 2003). The inhibitory effect of raw milk can indicate that *L. monocytogenes* grows more quickly in raw milk cheese than in cheese made from pasteurized milk. Factors that can limit growth are the LPS, lactic acid, competition for nutrients and steric limitations (Bemrah *etal*., 1998). Extrinsic parameters such as low pH or low water activity can also have an effect on the behavior of *L. monocytogenes* in white brined cheeses. In present study one *L. monocytogenes* isolate was recovered from pasteurized white brined cheeses samples using the conventional methods. *L. monocytogenes* in these products can be considered as important as in ready-to-eat products. It has been estimated that normal pasteurization processes can be effective in the destruction of this pathogen so conventional cooking could eliminate this organism (Norrung, 2000). MICROBACT system was designed to stimulate conventional biochemical substrate used for the identification of *Listeria* species giving the characterized species with percentage probability number using MICROBACT computer soft ware. Although conventional method of selective culture media and the Microbact™ System were designed to isolate and characterize *L. monocytogenes*.

The combination between conventional methods and PCR allow accurate detection of *L. monocytogenes* in many food samples products and considered as a very rapid screening technique (Sanaa, *et al*., 1993). Although conventional methods and the Microbact System were designed to isolate and characterize *L. monocytogenes*, however six of the isolates with typical characters for *L. monocytogenes* was proved to be as *L. monocytogenes* using primers targeting the (*hlyA*) gene by PCR

technique, from all the isolates of suspected *L. monocytogenes* the same six samples confirmed by biochemical test was confirmed by PCR technique. These findings are also reported by Pieter (Gouws and Liedemann, 2005) who found that PCR was able to eliminate the false positives and detect all *L. monocytogenes* in the food products. Selection of suspect colonies was also problematic. In addition to the fact that the incidence of non-pathogenic species of *Listeria* was higher than the incidence of *L. monocytogenes*, there was also the occurrence of *Listeria*-like organisms. These organisms had the typical appearance of *Listeria* on the selective medium, but were not *Listeria* species as confirmed by PCR. Phenotypic properties by which the bacteria are identified when using culture methods may not always be expressed and may be difficult to interpret or classify (Malorny *et al*., 2003). PCR is considered to be more reliable than conventional identification since it based on stable genotypic characteristics rather than the biochemical or physiological traits, which can be genetically unstable (Lawrence, and Gilmour, 1994). The potential for many types of foods to be cross-contaminated must be recognized, however, either directly or via surfaces and equipments that may become contaminated with *L. monocytogenes* after being in contact with raw foods (Mena *et al*., 2004). The purpose of this study was to determine the prevalence of white brined cheese contaminated with *L. monocytogenes* and PCR was employed to detect all *L. monocytogenes* strains in these products that showed high specificity in detection *L. monocytogenes* in tested white brined cheese samples. In an experiment conducted in Iran to determine the prevalence of *L. monocytogenes* in milk products, the final identification with PCR assay by using *prs* primers was considered a highly sensitivity 3.5 x 10 cfu/ml and specific method for

identification of the suspected colonies of *L. monocytogenes* in milk products (Jami *et al.,* 2010).

L. monocytogenes can be present in animal feed. It has been reportedly as a source of listeriosis in many cases in animal farm through the contamination that would happen in food chain (Fenlon, 1999) and for this reason animal products considered to be contaminated with *L. monocytogenes* but it might have *Listeria*-like characteristics on enrichment medias as blood agar. Several studies have been demonstrated how PCR help in rapid detection of *L. monocytogenes* in milk and milk products which their source from animal and to show the differentiate between suspect isolates that include non-haemolysed strains of *L. monocytogenes* and non-pathogenic strains by methods of ISO 1999. PCR was employed to solve the problem of interpretation of classical biochemical and serological typing in only one step without the need for additional examination (Holko *et al*., 2001). Since the isolates of *L. monocytogenes* from white brined cheeses were low, it appears that white brined cheeses products are subjected to cross-contamination during processing, handling and storage. Therefore, it is in must to take efficient preventive measures to limit the contamination of white brined cheeses and other food samples. Two factors are suggested to control contamination with *L. monocytogenes*.

Firstly, is the Hazard Analysis and Critical Control Point (HACCP) systems and Sanitation Standard Operating Procedures (Sanitation SOPs). Other food safety programs at the retail level applied to prevent cross-contamination and exposure to the pathogen (Holah *et al*., 2004). Secondly, storage time and temperature are other important factors in controlling the growth of *L. monocytogenes* by consumer awearnce of refrigerator temperature control and other factors (National Advisory Committee on Microbiological Criteria for Foods, 2005; Kosa *et al*., 2007).

The results of the present study demonstrated that there is a potential risk in these white brined cheeses products for consumer health in Jordan.

Although *L. monocytogenes* was isolated from some of the examined sample, *Listeria* species proved to be pathogenic to human in some cases. The actual incidence of listerosis is not officially recorded in Jordan, because *L. monocytogenes* is not included in most routinely performed medical test. A routine monitoring plan, including analysis of the product and environment for the presence of *L. monocytogenes* should be a part of national monitoring quality control program. Certain areas in the plant should receive special attention when evaluating the potential to contribute the morphological quality of the product. Contamination possibility in white brined cheeses products should not disregarded. The importance for hygiene conditions describe in Jordanian HACCP program should be enforced to minimize the growth of *L. monocytogenes* in white brined cheeses and food products during manufacturing, handling and storage process.

6. Conclusion and recommendations

- The presence of *L. monocytogenes* has been demonstrated for the first time in wide varieties of white brined cheese samples sold in Amman-Jordan.
- The morphological procedures for identification of *L. monocytogenes* is not successful because it depends on colony features, which can genetically be unstable and lacks accuracy.
- Molecular techniques have proven to be beneficial when working with food born pathogen because of it`s high specificity, sensitivity and rapidity
- It may be difficult to avoid *L. monocytogenes* in one or more steps of the food chain, from production to distribution, because the organism is so widespread in food plant environments
- The high bacteria contaminants detected in white brined cheeses products and undercooked food should be taken more seriously, since the potential risk of eating white brined cheese or undercooked foods is increasing.
- The Jordanian Standard Specification (JSS) lack regulation concerning *L. monocytogenes*, therefore, there is an urge to establish a national monitoring control for detecting *L. monocytogenes* in white brined cheeses.
- Establish a national record and documentation systems for outbreaks of food poisoning.
- There must be a corporation between research laborites and Jordanian food and drug administration (JFDA) as well as the ministry of health departments

7. References:

Almeida, P.F. and Almeida, R.C.C. (2000), A PCR protocol using *inl* gene as a target for specific detection of *Listeria monocytogenes*, **Food Control**, 2, 97-101.

Arnold, G.J. and Coble, J. (1995), incidence of *Listeria* species in foods in NSW, **Food Australia**, 47, 71-75.

Ayto, J (1990), the glutton's glossary*:* **Dictionary of Food and Drink Terms**, Routledge. p. 133.

Bansal, N.S. McDonell, F.H. Smith, A. Arnold, G. and Ibrahim, G. F. (1996), multiplex PCR assay for the routine detection of *Listeria* in food, **International Journal of Food Microbiology**, 33, 293–300.

Bemrah, N. Sanaa, M. Cassin, M.H. Griffiths, M.W. and Cerf O. (1998), quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk, **Preventative Veterinary Medicine**, 37,129-145.

Beumer, R.R. and Hazeleger, W.C. (2003), *Listeria monocytogenes*: Diagnostic problems. FEMS Immunol, **Medical Microbialogy**, 35, 191-197.

Bula, C.J. Bille, J. and Glauser, M.P. (1995), an epidemic of food-borne listeriosis in western Switzerland: Description of 57 cases involving adults, **Clinical disease,** 20, 66–72.

Carlos, V.S. Oscar, R.S. and Irma, Q.R.E. (2001), Occurrence of *Listeria* species in raw milk farms on the outskirts of Mexico City, **Food Microbiology**, 18, 177-181.

CDC (Center for Disease Control and Prevention), (2002), Public health dispatch: Outbreak of listeriosis — Northeastern United States, **Morbidity and Mortality Weekly Report,** 51, 950–951.

CDC. (1985), Epidemiologic notes and reports listeriosis outbreak associated with Mexican style cheese-California, **Morbidity Mortality Weekly Report** 34: 357-359 Center of diseases control. (2004), listeriosis.

Chao, G. Deng, Y. Zhou, X. Xu, Q. Qian, X. Zhou, L. and Zhu, B. (2006), prevalence of *Listeria monocytogenes* in delicatessen food products in China, **Food Control**, 17, 971-974.

Choi, W.S. and Hong, C.H. (2003), rapid enumeration of *Listeria monocytogenes* in milk using competitive PCR, **International Journal of Food Microbiology**, 84, 79-85.

Colak, H. Hampikyan, H. Bingol, E.B. and Ulusoy, B. (2007), prevalence of *Listeria monocytogenes* and *Salmonella* spp. in Tulum cheese, **Food Control**, 18, 576-579.

Cordano, A.M, Rocourt, J. (2001), occurrence of *Listeria monocytogenes* in food in Chile. **International Journal of Food Microbiology**, 70, 175-178.

Da Silva, M.C.D. Hofer, E. and Tibana, A. (1998), incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro, Brazil, **Journal of Food Protection,** 61, 543-356.

Davies, A.R. Capell, C. Jehanno, D. Nychas, G.J.E. and Kirby, R.M. (2004), Incidence of foodborne pathogens on European fish, **Food Control**, 12, 67-71.

Donnelly. (1999), Conventional Methods to Detect and Isolate *Listeria* monocytogenes. In: *Listeria,* **Listeriosis and Food Safety**, 2nd ed., E.T. Ryser, E.H. Marth (Eds.), Marcel Dekke Inc., New York pp. 225–260.

Dulbecco, R (1954), plaque formation and isolation of pure lines with poliomyelitis viruses, **International Journal of Experimental Medicine,** 99, p. 167-182.

European Commission. (2005), Commission regulation (EC) no. 2073/2005 of FDA **Bacteriological Analytical Manual**. Gaithersburg, USA: AOAC Int., Appendix. 3. 64.

Farber, J.M and Peterkin, P.I. (1991), listeria monocytogene as a food pathogen, **Microbiology Review**,55, 752-811.

Farber, J.M. Johnston, M.A. Purvis, U. and Loit, A. (1987), surveillance of soft and semi-soft cheeses for the presence of *Listeria* spp. **International Journal of Food Microbiology**, 5, 157-163.

Fenlon, D.R (1999), *Listeria monocytogenes* in the natural environment. In: Ryser, E.T., Marth, E.H. (eds.), *Listeria*, **Listeriosis and Food Safety**, 21–33.

Food and Drug Administration (FDA). (1999), **Bad bug book**: *Listeria monocytogenes*.

Center for Food Safety and Applied Nutrition. (1992), "The *Bad Bug Book*: **Foodborne Pathogenic Microorganisms and Natural Toxins Handbook.**

Fleming, D.W. M.D. Cochi, K.L. MacDonald and Brondum J. (1985), Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. **New England Journal of Medicine**, 312: 404-407.

Gaillard M. Maffeo, A. Penta, M. Petrone, G. (1986), detection of *Listeria monocytogenes* in cheese , **Food Control**, 12, 711-713.

Gameiro, N. Ferreira-Dias, S. Ferreira, M. Brito, L. (2007), Evolution of *Listeria monocytogenes* populations during the ripening of naturally contaminated raw ewe's milk cheese, **Food Control**, 18, 1258-1262.

Gasanov, U. Hughes, D. and Hansbro, P.M. (2005), **Methods for the Isolation and Identification**.

Gouws, P.A. and Liedemann, I. (2005), Detection of *Listeria monocytogenes* in food products, **Food Technology and Biotechnology**, 43, 201-205.

Greenwood MH, Roberts D, Burden P. (1991) The occurrence of *Listeria* species in dairy products: a national survey in England and Wales. **International Journal of Food Microbiology**, 12,197-206.

Griefiths, M. W. (1989), *Listeria monocytogenes*: Its importance in dairy industry**, Journal Science of Food and Agriculture,** 47, 133-158**.**

Guerra, M.M. McLauchlin, J. Bernardo, F.A. (2001), *Listeria* in ready-to-eat and underprocessed foods produced in Portugal, **Food Microbial**, 18, 423-429.

Holah, J.T. Bird, J. Hall, K.E. (2004), the microbial ecology of high-risk, chilled food factories evidence for persistent *Listeria* spp. and *Escherichia coli* strains, **Journal of Applied Microbial, 97, 68-77.**

Holko, J. Urbanova, M. Kantikova, K. Pastorova, V.Kmee (2001), State Veterinary Institute Dolnˇ Kubin Institute of Animal Physiology of Slovak **Academy of Sciences Kosice**.

ISO, (1996), Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method. International Standard ISO 11290-1. International Organization for Standarization. Geneva, Switzerland.

ISO, (2004), Modification of the isolation media and the haemolysis test, and inclusion of precision data. ISO 11290-1: 1996/ Amd 1: 2004. International Organization for Standarization. Geneva, Switzerland.

Jacquet, C. Gouin, E. Jeannel, D. Cossart, P. Rocourt, J. (2002), Expression of ActA, Ami, InlB, and listeriolysin O in *Listeria monocytogenes* of human and food origin, **Applied and Environmental Microbiology**, 68, 616–622.

Jalali, M. and Abedi, D. (2008), Prevalence of *Listeria* species in food products in Isfahan, Iran, **International Journal of Food Microbiology**, 122, 336–340.

James, S.M. Fannin, S.L. Agree, B.A. Hall, E. Parker, J. Vogt, J. Run, G. Williams, J. (1985), Listeriosis outbreak associated with Mexican-style cheese-California, **Morbidity Mortality Weekly Report**, 34, 357-359.

Jami, S. Jamshidi, A. and Khanzadi, S. (2010), **World Applied Science**,10, 249-253.

Jay, J.M. (2000), high-temperature food preservation and characteristics of thermophilic microorganisms. In: Jay, J.M. (eds.), **Modern Food Microbiology**. D. Van Nostrand Company, New York, pp. 341-362

Kabuki DY, Kuaye Y, Wiedmann M, Boor K. (2004), molecular subtyping and tracking of *Listeria monocytogenes* in Latin-style fresh-cheese processing plants, **Journal of Dairy Science**, 87, 2803–2812.

Klara, M. P., Ellen, R. W. (2009), Listeriosis. **Seminars in Fetal & Neonatal Medicine**, 33, 1-6.

Klein, P.G. and Juneja, V.K. (1997), Sensitive detection of viable *Listeria monocytogenes* by reverse transcription-PCR. **Applied Environment and Microbiology,** 63, 4441-4448.

Kosa, K.M., Cates, S.C., Karns, S., Godwin, S.L., Chambers, D., (2007), Consumer home refrigeration practices: Results of a web-based survey. **Journal of Food Protocol**, 70, 1640-1649.

Koutsoumanis, K., and A. Angelidis**.** (2007), Probabilistic modeling approach.

Lampel, K. A., P.A. Orlandi, L. Kornegay. (2000), Improved template preparation for PCR-based assays for detection of food-borne bacterial pathogens. **Applied Environment and Microbiology ,**66, 4539-4542**.**

Lawrenece, L. M., and A. Gilmour. (1994), Incidence of *Listeria* spp. And *Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR. **Applied Environment Microbiology,** 60, 4600-4604.

Longhi, C., Maffeo, A. Penta, M. Petrone, G. Seganti, L. and Conte, M.P. (2003), Detection of *Listeria monocytogenes* in Italian-style soft cheeses. **Journal of Applied Microbiology**, 94: 879–885.

MacGowan, AP, Bowker K, McLauchlin J, Bennett PM, Reeves DS. (1994), the occurrence and seasonal changes in the isolation of *Listeria* spp. in shop bought food stuffs, human faeces, sewage and soil from urban sources. **International Journal of Food Microbiology**, 21, 325-334.

Malorny B. Tassios P.T. Ridstrom P. Cook N. Wagner M. Hoorfar J. (2003): Standardization of diagnostic PCR for the detection of foodborne pathogens. **International Journal of Food Microbiology**, 83, 39–48.

Manfreda, G. CESARE de A. STELLA, S. COZZI, M. CANTONI, C. (2005) ,occurrence and ribotypes of *Listeria monocytogenes* in Gorgonzola cheeses. **International journal of Food Microbiology.**Amsterdam, v.10, .287-293.

Mead, P.S., Slutsker, L., Dietz, V., nMcCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauxe, R.V. (1999), **Foot-related Illness and Death in the United States**, 5, 607-625.

Mena, C., Almeida, G., Carneiro, L., Teixeira, P., Hogg, T., Gibbs, P.A., (2004), Incidence of *Listeria monocytogenes* in different food products commercialized in Portugal. **Food Microbiology**, 21, 213-216.

Mengaud, J., Vicente, M., Chenvert, J., Pereria, J.M., Geofroy, B., Gicquel-Sanzey, B. (1988), expression in *Escherichia coli* and sequence analysis of the listeriolysin O determinant of *Listeria monocytogenes*. **Infection and Immunity**, 56, 766-772.

Monge R, Utzinger D, Arias, ML. (1994) incidence of *Listeria monocytogenes* in pasteurised ice cream and soft cheese in Costa Rica. **Revista de Biologa Tropica**, 42, 327-328.

Murray, E.G.D., Webb, R.A., Swann, M.B.R., (1926), the Genus *Listeria* and *Listeria monocytogenes*: Phylogenetic Position, Taxonomy, and Identification. In: Ryser, E.T., Marth, E.H. (eds.), *Listeria*, **Listeriosis and Food Safety**, 2nd ed. Marcel Dekker Inc., New York, NY, pp. 1–3.

National Advisory Committee on Microbiological Criteria for Foods. (2005), considerations for establishing safety-based consume-by date labels for refrigerated ready-to-eat foods. **Journal of Food Protection**, 68, 1771-1775.

Nørrung, B. (2000), microbiological criteria for *Listeria monocytogenes* in foods under special consideration of risk assessment approaches. **International journal of Food Microbiology**, 62, 217–221.

Olivira, M.K (1993), detection of *Listeria monocytogenes* in brazillian minas cheese, **Food Microbiology**, 12, 65-67.

Pastorova, K., Holko, I., Urbanova, J., KantIkova, M., Kmee, V. (2001), direct detection of *Listeria monocytogenes* in milk and milk products by nested PCR. In: **Hygiena Alimentorum**, 145-146.

Pintado, C.M.B.S.; Oliveira, A.; Pampulha, M.E.; Ferreira, M.A.S.S. , (2005) prevalence and characterization of *Listeria monocytogenes* isolated from soft cheese. **Food Microbioogy**, 22, 79-85.

Pirie, J.H.H. (1927), new disease of veld rodents. " Tiger River Disease ". Publ. S. Afr. Inst. Med. Res. 3, 163-186.position, taxonomy, and identification. In: Ryser ET, Marth EH editors.

Powell, H. A. C. M. Gooding, S. D. Garrett, B. M. Lund, and R. A. McKee. (1994), Proteinase inhibition of the detection of *Listeria monocytogenes* in milk using the polymerase chain reaction. Letters, **Applied Microbiology**, 18, 59-61.

Ramos, S.N.M.; COSTA, C. A. (2003), ocorrência de *Listeria monocytogenes* em queijo artesanal tipo coalho comercializado na cidade de Manaus- AM, Brasil. **Acta Amazônica Manaus**, v.33, n.4, p.613-618.

Rocourt J. (1999), The geneus *Listeria* and *Listeria monocytogenes*: phylogenetic.

Rossen K. Fritsch, E.F., Maniatis, M (1988), molecular subtyping for detection *Listeria* in dairy products, **Food Protection**, 5, 11-13

Rudolf, M., & Scherer, S. (2001). High incidence of *Listeria monocytogenes* in European red smear cheese. **International Journal of Food Microbiology**, 63, 91–98.

Sado, P.N., Jinneman, K. C., Husby, G. J., Sorg, S. M., Omiecinski, C. J. (1998), Identification of *listeria monocytogenes* from unpasteurised apple juice using rapid test kits. **Journal of Food Protection**, 61, 1199-1202.

Sambrook, J., & Russel, D. W. (2000), molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor, NY, USA: Cold Spring Harbor Press.

Sambrook, J., Fritsch, E.F., Maniatis, M., (1989), Molecular cloning, a Laboratory Manual, 2nd ed. NY: Cold Spring Harbor Laboratory Press.

Sanaa M, Coroller L, Cerf O. (1993) Risk assessment of Listeriosis linked to the consumption of two soft cheeses made from raw milk: **Camembert of Normandy and Brie of Meaux. Risk Analysis**, 24,389-399.

Schlech W.F., Lavigne P.M., Borrolussi R.A., Allen A.C. (1983), epdimic listeriosis evidence for mission by food. **New England**, 308, 203-206.

Schlech, W.F., (1997), *Listeria* gastroenteritis-old syndrome, new pathogen. **New England**, 336, 130-132.

Schuchat, A., Swaminathan, B., Broome, C.V., (1991), epidemiology of human listeriosis. **Clincal Microbiology Review**, 4, 169-183.

Schwab, J.P.(1994), *Listeria monocytogenes em queijo colonialartesanal comercializado em Porto Alegre*. **Porto Alegre**,. 101p. [Dissertação de Mestrado. Agronomia. Universidade Federal do Rio Grande do Sul.

Souza, R.A.(2002), Incidência de L. monocytogenes em queijo tipo coalho artesanal comercializado à temperature ambiente em Fortaleza - CE. Fortaleza,. 78p. [Dissertação de Mestrado. Departamento de Tecnologia de Alimentos. Universidade Federal do Ceará].

Swaminathan, B. (2001), foodborne pathogenic bacteria: *Listeria monocytogenes*, In: Doyle, M.P., Beuchat, L.R., Montville, T.J. (eds.), **Food Microbiology**, 77, 337–352.

Tamime, A.Y. (1991). R. K. Robinson and A. Y. Tamime. *Listeria monocytogenes* in fresh and processed foods**, Feta and Related Cheeses**. Wood head publishing, 12, 71-77

USDA, (U.S. Department of Agriculture), (2006), re call Information Center.

Varnam, A.H. and Evans. M.G. (1991), foodborne Pathogens: Illustrated Text. 1st Edn., **Mosby Elsevier Health Science**,10, pp: 557.

Vazquez-Boland, J.A. Kocks, C.M. Dramsi, S. Ohayon, H. Geoffroy, C. Mengaud, J. and Cossart, P. (1992), Nucleotide sequence of the lecithinase operon of *Listeria monocytogenes* and possible role of lecithinase in cell-to-cell spread. **Infection and Immunity,** 60, 219–230.

Vernozy-Rozand. (1998), **International Journal of Food Microbiology** 64 217 –221.

Vitas, A.I. Aguado, V. and Garcia-Jalon, I. (2004), Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). **International Journal of Food Microbiology**, 90, 349–356.

Vlaemynck, G., Lafarge, V., Scotter, S., (2000), Improvement of the detection of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium. **Journal of Applied Microbiology**, 88, 430-441.

Wernars, K., C. J. Heuvelman, T. Chakraborty, and S. H. W. Notermans. (1991), Use of the polymerase chain reaction for direct detection of *Listeria monocytogenes* in soft cheese. **Journal of Applied Bacterioliogy**, 70:121-126. 3418.

Winter, P. Schilcher, F. Bago, Z. Schoder, D. Egerbacher, M. Baumgartner, W., and Wagner, M., (2004), Clinical and histopathological aspects of naturally occurring mastitis caused by *Listeria monocytogenes* in cattle and ewes. **Journal of Veterinary medicine,** 51, 176-179.

Zhou, X. Jiao, X. (2005), Polymerase chain reaction detection of *Listeria monocytogenes* using oligonucleotide primers targeting *actA* gene. **Food Control**, 16, 125-130.

8. Appendices:

8.1 Appendix A: culture media and reagents

Half Fraser Broth Base (Oxoid, England):

contained in 1000 ml distilled water: proteose peptone 5 gm, tryptone 5 gm, 'lab-lemco' powder 5 gm, yeast extract 5 gm, sodium chloride 20 gm, di-sodium hydrogen phosphate 12 gm, potassium dihydrogen phosphate 1.35 gm, aesculin 1 gm, and lithium chloride 3 gm. pH was adjusted to 7.2±0.2. After autoclaving at 121º C for 15 minutes, medium was cooled to 50º C then one vial of Half Fraser Selective Supplement SR 0166E which is composed of; ferric ammonium citrate 112.5mg, nalidixic acid 2.25mg, and acriflavine 2.8125mg dissolved in 4 ml of 1:1 ethanol : sterile distilled water were added to each 225 ml medium.

Fraser Broth Base (Oxoid, England):

contained in 1000 ml distilled water: proteose peptone 5 gm, tryptone 5 gm, 'lab-lemco' powder 5 gm, yeast extract 5 gm, sodium chloride 20 gm, di-sodium hydrogen phosphate 12 gm, potassium dihydrogen phosphate 1.35 gm, aesculin 1 gm, and lithium chloride 3 gm. pH was adjusted to 7.2±0.2. After autoclaving at 121º C for 15 minutes, medium was cooled to 50º C then one vial of Fraser Selective Supplement SR 0156E which is composed of; ferric ammonium citrate 250mg, nalidixic acid 10mg, and acriflavine 12.5mg dissolved in 5 ml of 1:1 ethanol : sterile distilled water were added to each 500 ml medium.

Listeria Selective Agar Base (Oxford Formulation) (Oxoid, England):

contained in 1000 ml distilled water: Colombia blood agar base 39 gm, aesculin 1 gm, ferric ammonium citrate 0.5 gm, lithium chloride 15 gm. pH was adjusted to 7.2±0.2. After autoclaving at 121º C for 15 minutes, medium was cooled to 50º C then one vial of Listeria Selective Supplement (Oxford Formulation) SR0140E which composed of; cycloheximide 200mg, colistin sulphate 10mg, acriflavin 2.5mg, ceftotetan 1mg, and fosfomycin 5mg dissolved in 5 ml 70% ethanol, were added to each 500 ml medium.

Chromogenic Listeria Agar (ISO) Base (Oxoid, England):

contained in 1000 ml distilled water: enzymatic digest of animal tissues 18 gm, enzymatic digest of casein 6 gm, yeast extract 10 gm, sodium pyruvate 2 gm, glucose 2 gm, magnesium glycerophosphate 1 gm, magnesium sulphate (anhydrous) 0.5 gm, sodium chloride 5 gm, lithium chloride 10 gm, di-sodium hydrogen phosphate (anhydrous) 2.5 gm, x-glucoside mix 0.05 gm, and agar 12 gm. pH was adjusted to 7.2±0.2. After autoclaving at 121º C for 15 minutes, medium was cooled to 50º C then one vial of Chromogenic Listeria Selective Supplment (ISO) SR0226E which composed of; nalidixic acid 10mg, polymyxin B 38.35mg, amphotericin 5mg, and ceftazidime 10mg dissolved in 2 ml sterile distilled water, and also one vial of Brilliance™ Listeria Differential Supplement SR0228E which composed of lecithin solution 20 ml, were added to each 480 ml. Motility Test Medium (Himedia, India) contained in 1000 ml distilled water: tryptose 10 gm, sodium chloride 5 gm, and agar 5 gm (pH was adjusted to 7.2±0.2).

Sheep Blood Agar Base (Oxoid, England):

contained in 1000 ml distilled water: tryptone 14 gm, peptone 4.5 gm, yeast extract 4.5 gm, sodium chloride 5 gm, and agar 12 gm. pH was adjusted to 7.3±0.2. After autoclaving at 121º C for 15 minutes, medium was cooled to 50º C then 7% of sterile sheep blood was added aseptically.

Tryptone Soya Agar (Oxoid, England):

contained in 1000 ml distilled water: tryptone 15 gm, soya peptone 5 gm, sodium chloride 5 gm, and agar 15 gm (pH was adjusted to 7.3±0.2).Yeast Extract powder (Oxoid, England) (pH was Tryptone Soya Broth (Oxoid, England) contained in 1000 ml distilled water: pancreatic digest of casein 17 gm, enzymatic digest of soybean 3 gm, sodium chloride 5 gm, di-potassium hydrogen phosphate 2.5 gm, and glucose 2.5 gm (pH was adjusted to 7.3±0.2).

8.2 Appendix B:

Stainless steel surgical spatula, forceps, and handle (Swann-Morton, England).
Stomacher bags (Seward, UK).
Sterile disposable serological graduated pipettes (1ml, 2ml, 5ml, and 10ml, Europe)
Sterile plastic Petri dishes (94mm/16mm) (Greiner bio-one, Austria).
160 mmX15mm autoclave test tubes with screw caps.
250 ml autoclave bottles (Duran, Germany).
500 ml autoclave bottles (Duran, Germany).
1000 ml autoclave bottles (Duran, Germany).
Digital top loading balance (ADAM, ACB Plus-300, U.K).
PH-meter (Hanna, pH 211, U.K).
Water Distiller (GFL, 2008, Germany).
Steam sterilizer (Raypa, AES-75, Spain).
Autoclave (smeg, HV-50L, Japan).
Biosafety cabinet (BIOSTAR Level-2, Spain).
Stomacher circulator unit 400 (Seward, UK).
Incubator 30º C±1 (P-Selecta, Spain).
Incubator 37º C±1 (memmert, Germany).
Incubator 25º C±1 (memmert, Germany).
Water bath with shaker (memmert, Germany).
Digital Vortex mixer (VELP Scientifica, Europe).

تحديد و تشخيص جراثيم الليستيريا مونوسايتوجينز المعزولة من الاجبان المملحة البيضاء باستخدام الطرق التقليدية البيوكيميائية و الجزيئية PCR

اعداد
رنا عاطف محمد قاسم

المشرف
الدكتور مهند وليد العكش

المشرف المشارك
الدكتور حمزة مرعي القادري

تمت الدراسة لتحديد وتشخيص جراثيم الليستيريا مونوسايتوجينز المعزولة من الاجبان المملحة البيضاء في عمان – الاردن باستخدام الطرق التقليدية البيوكيميائية و تأكيد تشيخصها باستخدام الطريقة الجزيئية PCR.

تم جمع 250 عينة من الاجبان المملحة البيضاء (عزلت هذة العينات من (70) عينة عكاوي ، (63) عينة حلوم ، (40) جبنة مبسترة ، (32) جبنة مغلية و (45) جبنة نابلسية) من اسواق عمان المحلية ، طبقت طريقة الهيئة الدولية للمعايير القياسية ISO باستخدام طريقة العزل التقليدية ذات المرحلتين التي تتطلب مرحلة التنشيط البدائية ومن ثم مرحلة التنشيط الثانية للعينات والتي تنتهي بالتشخيص الافتراضي. أظهرت نتائج طريقة العزل التقليدية تلوث 28 عينة (11.2) من اصل 250 عينة بجراثيم الليستييريا حيث كانت اعلى نسبة تواجد (14.28) في عينات العكاوي. عزلت جراثيم الليستيريا مونوسايتوجينز من 6 عينات (3 عينات عكاوي، 1 عينة حلوم ، عينة نابلسية ، عينة مبسترة و 0 عينة مغلية). أبدت نتائج التقنية الجزيئية تشخيص العينات الست الملوثة ، و ان الانتقال من طرق التشخيص التقليدية الى طرق التشخيص الجزيئية تعد ضرورية لتميز الاخيرة بالدقة العالية. ان النتائج الممثلة في هذه الدراسة تبين الخطورة المحتملة من تلوث الاغذية بجراثيم الليستيريا مونوسايتوجينز.